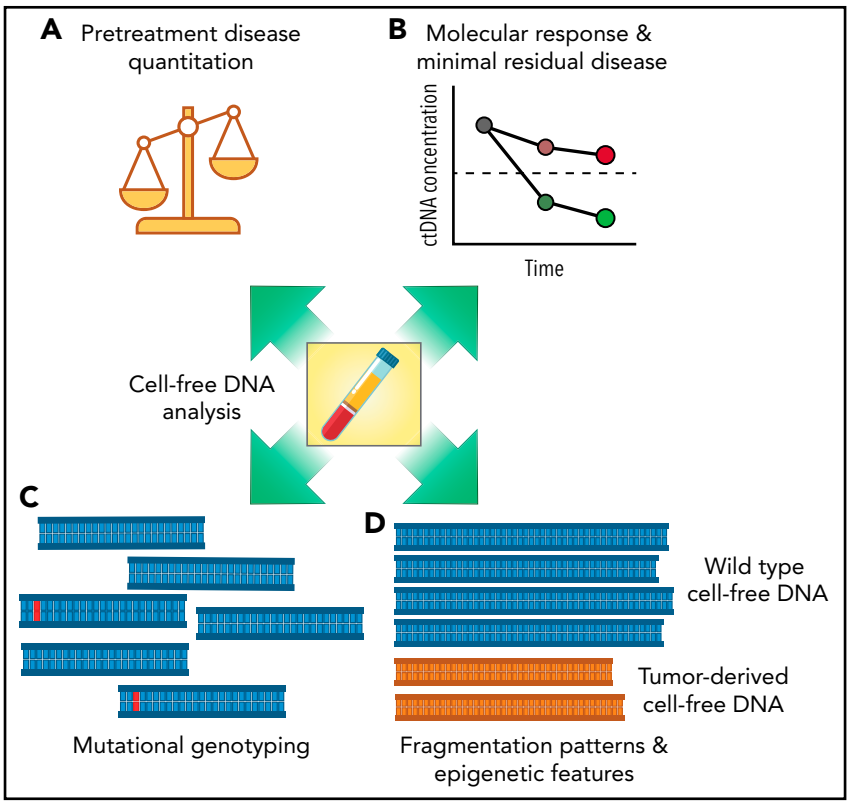
Comment on Meriranta et al, page 1863

The many facets of liquid biopsies in lymphoma

David M. Kurtz | Stanford University

The study from Meriranta et al[1] in this issue of *Blood* details circulating tumor DNA analysis from 101 patients with large B-cell lymphomas undergoing first-line therapy on the Nordic LBC-05 trial. Using a custom capture-based sequencing approach, the authors assessed plasma cell-free DNA at time points before, during, and after immunochemotherapy. The investigators extensively analyze diverse features of tumor-derived cell-free DNA molecules, including quantitative measurements of tumor DNA, dynamic measurements during and after therapy, and mutational genotypes.

In addition to uses for tumor-burden assessment and mutational profiling, the authors explore fragmentation patterns of mutant and wild-type cell-free DNA molecules across these same samples. This study meticulously assesses the clinical and prognostic significance of each of these features of cell-free DNA. Specifically, the authors convincingly demonstrate the adverse prognostic value of high circulating tumor DNA levels before therapy and detectable residual circulating tumor DNA at the end of therapy. Moreover, they demonstrate the performance of circulating tumor DNA analysis to identify tumor-specific genotypes, including associations between *TP53* mutation and adverse outcomes, as well as emergence of occult *MYC* genomic events in relapsing disease.



Multiple dimensions of cell-free DNA in lymphomas. The study from Meriranta et al examines multiple aspects of cell-free DNA in large B-cell lymphomas, including pretreatment disease quantification (A), molecular response assessment (B), mutational genotyping (C), and DNA fragmentation patterns (D). Each feature of cell-free DNA molecules has potential for translation into the clinic as a prognostic biomarker.

The utility of circulating tumor DNA in the clinic for mutational genotyping, pretreatment prognostication, and molecular response assessment has been an important topic in the last 5 years. Initial studies used immunoglobulin high-throughput sequencing to quantify disease burden,[2,3] whereas more recent studies, such as the current work by Meriranta et al, used capture-based sequencing to both quantify disease burden and identify tumor-specific mutations.[4-6] This study adds to the growing body of evidence that circulating tumor DNA can provide useful prognostic information before treatment,[4,7] assess molecular response at interim time points and residual disease at the end of therapy,[3-6] and identify tumor-specific mutations.[5,6] Furthermore, recent studies have suggested differences in the length of DNA fragments derived from tumor vs nontumor origin, with shorter DNA fragments enriched for tumor-derived molecules.[8] The present study extends this finding to large B-cell lymphomas, demonstrating robust and reproducible patterns in molecular length of plasma DNA from patients with lymphoma.

The work from Meriranta et al further clarifies the concept that circulating tumor DNA is not a single assay but rather an analyte that can reveal multiple dimensions of a tumor (see figure). Each of these features potentially exposes independent aspects of a malignancy, such as tumor burden, responsiveness to therapy, and underlying biological and molecular differences, which may serve as actionable biomarkers for targeted treatments. As the field becomes more refined, specifying which of these features are of most interest—and which assays are best suited to each application—will become important. Methods to detect and quantify pretreatment and residual tumor burden have been extensively studied previously. Technologies for quantifying circulating tumor DNA burden are therefore closest to translation, with clinical trials focused on risk-adapted therapy for diffuse large B-cell lymphoma (DLBCL) a possible path forward. Methods to identify specific mutations or mutational subtypes also show significant promise, although clinical translation will depend on the development of subtype-specific targeted therapies for

treatment selection. Finally, methods to assess epigenetic tumor features in the absence of mutations, either through DNA fragmentation patterns or methylation,[9,10] have been the least studied in lymphomas, although potentially have the most promise for understanding the transcriptional programming of a given patient's disease.

It remains to be seen if the value of circulating tumor DNA in the clinic will primarily be as a "minimal residual disease" test, or whether techniques focused on characterizing the tumor genome or epigenome will become a routine part of clinical evaluation. However, each additional study further confirming and adding to the utility of liquid biopsies suggests that circulating tumor DNA analysis may become a common thread throughout a patient's experience with large B-cell lymphoma, augmenting or even replacing standard pathology or radiographic imaging. Ultimately, prospective clinical studies will be required to define how and when this analyte should be used to improve patient outcomes. Still, with the growing body of evidence for its utility, it is likely not a matter of if, but when, circulating tumor DNA becomes part of routine evaluation of DLBCL.

Conflict-of-interest disclosure: D.M.K. has served as a consultant for Roche and Genentech, has ownership equity in Foresight Diagnostics, and has patents pending related to methods for analysis of cell free nucleic acids and methods for treatment selection based on statistical frameworks of clinical outcome. ■

REFERENCES

1. Meriranta L, Alkodsi A, Pasanen A, et al. Molecular features encoded in the ctDNA reveal heterogeneity and predict outcome in high-risk aggressive B-cell lymphoma. *Blood*. 2022;139(12):1863-1877.
2. Kurtz DM, Green MR, Bratman SV, et al. Noninvasive monitoring of diffuse large B-cell lymphoma by immunoglobulin high-throughput sequencing. *Blood*. 2015;125(24):3679-3687.
3. Roschewski M, Dunleavy K, Pittaluga S, et al. Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. *Lancet Oncol*. 2015;16(5):541-549.
4. Kurtz DM, Scherer F, Jin MC, et al. Circulating tumor DNA measurements as early outcome predictors in diffuse large B-cell lymphoma. *J Clin Oncol*. 2018;36(28):2845-2853.
5. Rossi D, Diop F, Spaccarotella E, et al. Diffuse large B-cell lymphoma genotyping on the liquid biopsy. *Blood*. 2017;129(14):1947-1957.
6. Scherer F, Kurtz DM, Newman AM, et al. Distinct biological subtypes and patterns of genome evolution in lymphoma revealed by circulating tumor DNA. *Sci Transl Med*. 2016;8(364):364ra155.
7. Alig S, Macaulay CW, Kurtz DM, et al. Short diagnosis-to-treatment interval is associated with higher circulating tumor DNA levels in diffuse large B-cell lymphoma. *J Clin Oncol*. 2021;39(23):2605-2616.
8. Mouliere F, Chandrananda D, Piskorz AM, et al. Enhanced detection of circulating tumor DNA by fragment size analysis. *Sci Transl Med*. 2018;10(466):eaat4921.
9. Shen SY, Singhania R, Fehringer G, et al. Sensitive tumour detection and classification using plasma cell-free DNA methylomes. *Nature*. 2018;563(7732):579-583.
10. Snyder MW, Kircher M, Hill AJ, Daza RM, Shendure J. Cell-free DNA comprises an in vivo nucleosome footprint that informs its tissues-of-origin. *Cell*. 2016;164(1-2):57-68.

DOI 10.1182/blood.2021015022

PLATELETS AND THROMBOPOIESIS

Comment on Chen et al, page 1878

It takes guts to boost platelet reactivity and inflammation

Julie Rayes | University of Birmingham

In this issue of *Blood*, Chen et al identified the intestinal IL-33–ST2–serotonin axis as a key immune-endocrinal crosstalk that drives platelet reactivity during hemostasis and platelet-dependent neutrophil recruitment during inflammation.[1]

The gut is the largest endocrine organ in the body able to sense environmental cues to regulate intestinal homeostasis and host defense. A breakdown in the intestinal homeostasis can lead to inflammatory bowel diseases (IBDs), such as Crohn's disease and ulcerative colitis, which are associated with increased platelet reactivity and increased susceptibility to thromboembolic complications.[2]

The intestinal epithelium is a heterogeneous tissue comprised of multiple cell types, including neuroendocrine and intestinal epithelial cells (IECs). Enterochromaffin (EC) cells are a subset of intestinal enteroendocrine cells with potent mechano-sensors that are able to translate environmental signals to produce endocrine molecules such as the neurotransmitter and hormone serotonin (5-hydroxytryptamine [5-HT]). More recently, immune-driven signals released from IECs were also shown to stimulate the secretion of 5-HT from EC cells.[3] In response to intestinal damage or stress, IECs secrete cytokines such as interleukin-33 (IL-33), regulating intestinal homeostasis and immune responses.

IL-33, a member of the IL-1 family, is constitutively expressed in endothelial, stromal, and epithelial cells. IL-33 is present in the gut in steady state and its expression and release from IECs are increased during cell necrosis and tissue damage. In contrast to cell necrosis, IL-33 is inactivated during cell apoptosis by caspases, which limits an inflammatory response during programmed cell death. IL-33 acts as an alarmin by binding to its receptor ST2 expressed on hematopoietic and nonhematopoietic cells and participates in mucosal homeostasis and host defense. IL-33 supports allergic responses on multiple barrier sites, including the intestinal epithelium, by promoting type 2 immune response.[4]

Recently, Chen et al identified IL-33 released from necrotic IECs as a key trigger for quick serotonin release from EC cells, leading to gut mobility and peristaltic movement which facilitates the expulsion of parasites.[3] EC cells are the major source of peripheral 5-HT, and the release of 5-HT supports epithelial secretion, platelet function, peristalsis, wound healing, neutrophil recruitment to the site of inflammation,[5] and mucosal immunity. Serotonin